

REVIEW

Interaction of nanoparticles with biological systems

István Zoltán Vass, Zsuzsanna Deák, Kenny Paul, Sándor Kovács, Imre Vass*

Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

ABSTRACT Nanoparticles (NPs) are literally and figuratively infiltrating all fields of biological research. They are sophisticated tools that can be customized, either by smart engineering or by the attachment of specific ligands, to match the requirements of a particular task. Through their inherent and functionalized properties they are the basis for new developments while enhancing the efficiency of already existing techniques or rendering methods to be more specific. They provide new approaches for therapeutic applications and brand new platforms for diagnostic processes. In this review we provide an insight into the practical applications of NPs, emphasizing their use in biosensing, bioimaging, biomolecule delivery systems and enzyme immobilization. Since the interest in the interactions of NPs and biological systems is fairly new, we also elaborate on the drawbacks of their practical applications by reporting their potential toxicity in *in vitro* and *in vivo* systems.

Acta Biol Szeged 59(Suppl.2):225-245 (2015)

KEY WORDS

bioimaging
biosensing
functionalization
enzyme immobilization
nanoparticles
nanotoxicity

Introduction

According to the semi-standardized definition nanoparticles (NPs), either engineered or produced naturally, are particulate matter with at least one dimension of 100 nm or less. The defining characteristic of NPs is their size (Fig. 1), which is larger than individual atoms or molecules, but smaller than for example the influenza A virus, which has a diameter of 100-120 nm (Sharp et al. 1945). The properties of materials at nanoscale are typically different from those of bulk materials for two main reasons: one is the relatively large surface area of NPs, and the other is that below a certain size particles are subject to quantum phenomena. The most obvious consequence of reducing the size of particles is the concomitant increase in surface area to volume ratio, which leads to the very high reactivity of NPs, since the greater proportion of atoms or molecules are displayed on the surface rather than the interior of the material (Nel et al. 2006; Oberdörster et al. 2005). Depending on the type of application the increased chemical and biological reactivity may be positive and desirable (e.g., antioxidant activity, carrier capacity for drug and gene delivery, penetration of cellular barriers), but it can also enhance the negative and undesirable effects (e.g., toxicity, induction of oxidative stress) (Oberdörster et al. 2005).

The estimated global annual production of SiO₂ NPs is 5500 tons, and among others 3000 tons of TiO₂ NPs and 300

tons of carbon nanotubes are produced as well (Bondarenko et al. 2013). Most of these are not manufactured exclusively for research purposes as engineered NPs are increasingly being used in numerous consumer products, such as health and fitness, home and garden, food and beverage, electronics, computers, etc.

In recent years the use of NPs exponentially gained interest in numerous research fields, and generated momentum for the study of their interaction with biological systems. Here we provide an insight into the successful applications of NPs in bioimaging and biosensing, in clinical therapy and diagnostics. NPs are also present in agriculture in terms of research, as well as in terms of practical applications. We also elaborate on the drawbacks of NP use, due to their potential toxicity in *in vitro* and *in vivo* systems.

Engineering and production of NPs

NPs are formed in nature, produced via anthropogenic activities and engineered for particular purposes. In each of these events the particles are formed either through the disintegration of bulk material, which is called a top-down formation, or through the assembly of even smaller particles, which is a bottom-up process. In the following sections we provide a few examples of the formation of such particles.

Top-down formation of NPs

A straightforward example of a top-down production process

Submitted May 20, 2015; Accepted Sept 4, 2015

*Corresponding author. E-mail: vass.imre@brc.mta.hu

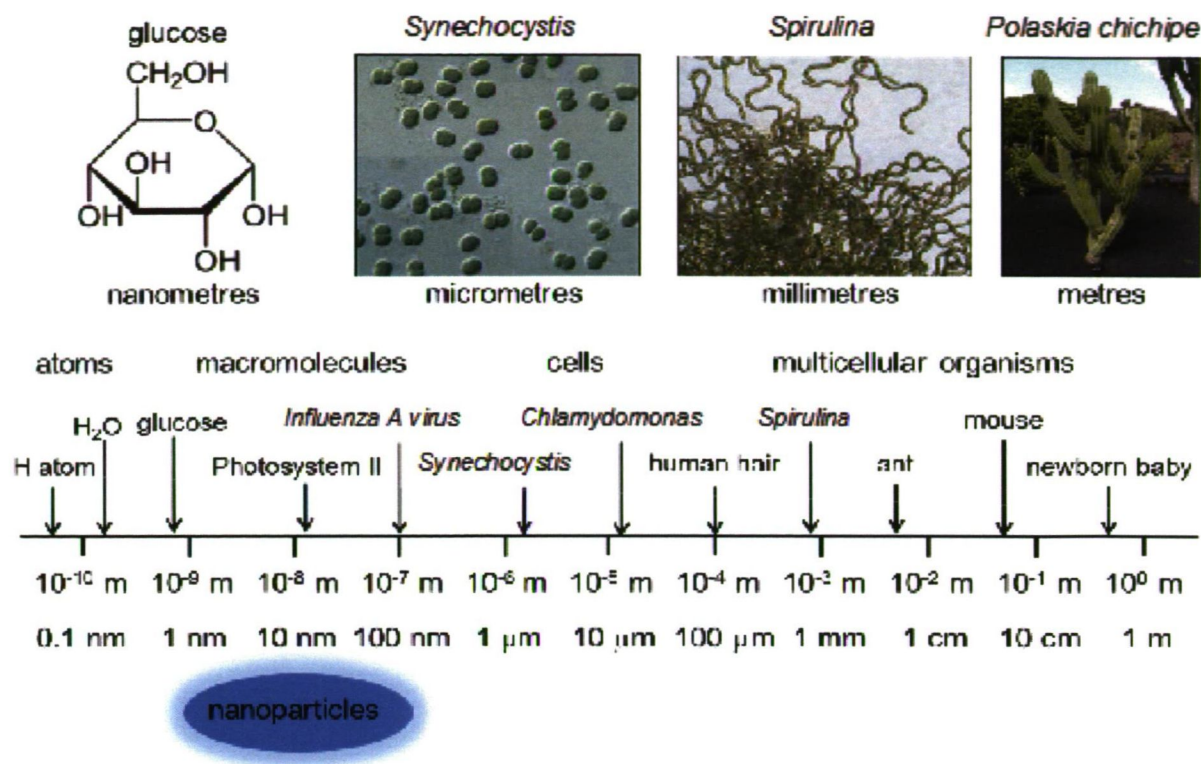


Figure 1. Logarithmic scale from meters to the tenth of nanometers to show the size of several biological objects and nanoparticles.

from nature is volcanic activity (Tepe and Bau 2014). This creates an array of metal (*e.g.*, Fe, Ca, Hg, Al, Co), silicon and carbon NPs. Human activities, such as wood and fuel burning also introduce a significant amount of NPs into the atmosphere (Hata et al. 2014). Mining, steel production and steel modification constitute a large source of NPs as well (Zimmer and Biswas 2001). NPs are not only produced at industrial levels, they are constantly formed around us and even laser printer cartridges emit NPs (Wang et al. 2011).

Top-down methods applied for the production of first generation NPs are various forms of milling and metal attrition. In these processes particles are removed from bulk material. A serious drawback of this method, besides having little control over tuning and controlling the chemical composition, is that the size and shape of the resulting particles show high variability.

Laser ablation is a top-down physical process, during which a small quantity of material is removed from the surface of any bulk material, forming an ablation plume, which is then deposited on specific substrates (Batani et al. 2013). Size, aggregation and yield efficiency of NPs may be controlled by adjusting the frequency and intensity of the laser pulses. This being a one-step method is considered a fast and cheap technique that produces fairly high purity NPs.

Nanosphere lithography is a mix of top-down and bottom-up approaches, and an effective tool to achieve high monodispersity (Colson et al. 2013). In a first step a flat surface is covered with hydrophilic and monodisperse spherical colloids (*e.g.*, polystyrene). This process forms a colloidal crystal mask on the respective surface, after which the desired material (*e.g.*, Au) is evaporated onto the crystal mask and will be deposited in the interstices of the spheres. By removing the mask in a last step, monodisperse nanodots are left behind on the flat surface. Depending on the material and production conditions an extra step might be necessary, in which the nanodots are crystallized (Fig. 2).

Bottom-up formation of NPs

In order to have adequate control over the main parameters of NPs bottom-up production techniques are favored, within which there are two major directions. The first requires reagents and precision equipment (*e.g.*, spray pyrolysis, inert gas condensation, UV-irradiation, laser ablation, nanosphere lithography, sol-gel fabrication, ultrasonic bath) while the second branch relies on the ability of various organisms (*e.g.*, bacteria, yeast, fungi, plants) to take up and form nano-sized aggregates/crystals from certain molecules.

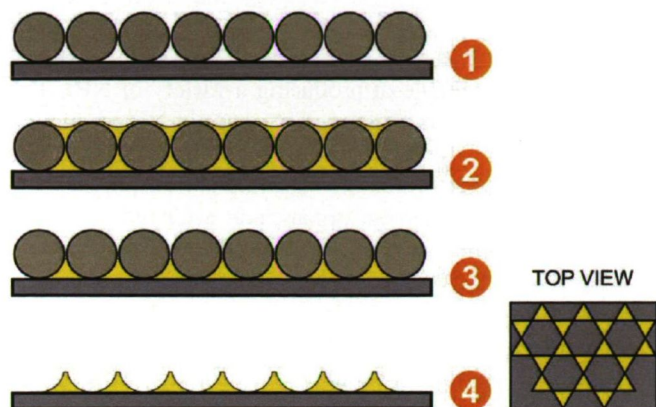


Figure 2. Nanosphere lithography. (1) Polystyrene spheres are immobilized on a flat surface, forming a mask. (2) Metal vapor fills the interstices of the mask. (3) Mask is removed from the setup. (4) Triangular nanoparticles are left behind.

Spray pyrolysis is a physical method to produce NPs. This is an aerosol process in which a solution, with an optimized concentration of solutes, is atomized following which the resulting droplets are heated (up to 1200 °C) and precipitated to produce solid particles (Fig. 3). This method is widely used to prepare metal oxide NPs, such as ZnO and is regarded as a low cost and high purity yielding technique (Ghaffarian et al. 2011).

Inert gas condensation is also a well-established physical method to produce metallic (e.g., Fe, Mn) NPs (Silva et al. 2014; Ward et al. 2006). A metallic source is evaporated using various heating processes in a closed chamber filled with a cooled and purified inert gas (e.g., He, Ar), at a low pressure. After the metal vapor condenses in the cool gas environment the particles will be collected on the cooled structural surface.

UV radiation is another effective way to yield monodisperse NPs (Stipe et al. 2004). In this process high intensity UV radiation is applied on combustion-generated soot particles and depending on the fluence and repetition rate of the irradiation, the size and morphology of NPs can be modulated (Fig. 4). Another approach is to form NPs by irradiating solutes (e.g., AgNO_3) stabilized in gelatine (Darroudi et al. 2011) or aqueous solutions (Dong et al. 2004) of a particular material (e.g., HAuCl_4), with UV light, which then reduces the respective solutes into NPs.

Sol-gel fabrication starts with the formation of a solution with solid particles (e.g., $\text{Fe}(\text{NO}_3)_3$). This sol phase then undergoes a gelation process via polycondensation or polyesterification. The resulting gel is aged and dried to remove the liquid phase and compact it. The dehydrated, dense gel is then decomposed at high temperatures and nano-sized powder is harvested from the process. It is applied in the production of NPs like Fe_2O_3 or silica (Moncada et al. 2007).

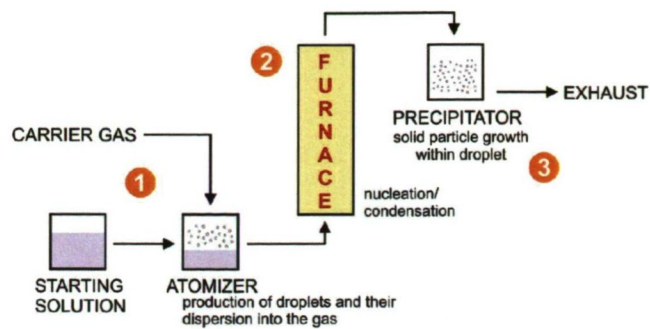


Figure 3. Spray pyrolysis. (1) A starting solution of the desired material is atomized while mixing it with a carrier gas. (2) The resulting droplets are sent through a furnace to achieve controlled condensation. (3) The condensed droplets are precipitated until solid particles are formed.

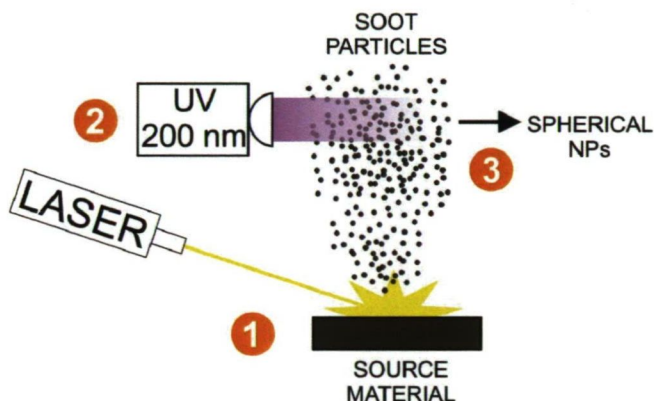


Figure 4. UV radiation. (1) Soot particles are formed through exposing bulk material to high temperatures. (2) The resulting particles are irradiated with high energy UV light. (3) Reduction of the soot particles occur and nanoparticles are formed.

Ultrasonic bath is a simple chemical method to produce Au NPs. An aqueous solution of HAuCl_4 is placed in an 80 °C water bath and is exposed to ultrasonication for a short time. Sodium citrate is then added to the solution which reduces the HAuCl_4 and forms aggregations of primary particles. A second, longer round of ultrasonication breaks apart these aggregates and forms highly monodisperse, spherical Au NPs (Lee et al. 2012).

NP formation via biological processes

Organisms are also able to produce NPs. Formation of NPs, their size, shape, quantity, monodispersity and other features are generally influenced by substrate concentration, exposure time to the respective substrate, environmental pH, temperature, and nutrient conditions. NP production by organisms gained momentum in the recent decade, the main reason being that it does not require hazardous reagents, it is cheap, fairly

simple and in general ecofriendly.

Bacteria

Actinobacter strains have the ability to extracellularly produce TiO_2 and ZnO NPs. For this to happen *Actinobacter* cultures are inoculated with K_2TiF_6 and zinc acetate, and incubated under normal culturing conditions for 48 hours (Singh et al. 2010). Intracellular synthesis of silver NPs using *Streptomyces* and *Streptoverticillium* strains is achieved by exposing these organisms to a certain (3.5 mM) concentration of AgNO_3 (Udaya Prakash et al. 2014).

Yeast

The yeast species *Candida glabrata* is able to intracellularly form CdS nanocrystals (Dameron et al. 1989). To achieve this aqueous solution of CdCl_2 is added to its culture medium before inoculation. The actual process of forming the NPs is a sequestration mechanism in order to remove Cd ions from the cells.

Fungi

If aqueous solution of AgNO_3 is added to the culture medium of the fungus *Verticillium*, the mycelia will produce 25 ± 12 nm sized Ag NPs intracellularly (Mukherjee et al. 2001). Intracellular production, however, means that one needs to harvest NPs from biomass, which is a complicated process. In an effort to avoid this complication using the pathogenic *Fusarium oxysporum* has opened up a fungal/enzyme-based *in vitro* approach for extracellular NP synthesis. The typical production process consists of adding the aqueous solution of the desired substrate (e.g., AgNO_3 , HAuCl_4 , SiF_6^{2-} , TiF_6^{2-}) to the mycelia-containing culture, and incubate the mix for an optimized length of time. During the process the fungus secretes various enzymes that either reduce, hydrolyze or alter the substrates in other ways. This setup has been used to produce several kinds of NPs, like Au NPs (Thakker et al. 2013), SiO_2 and TiO_2 NPs (Bansal et al. 2005) just to name a few.

Cyanobacteria

The filamentous cyanobacterium *Plectonema boryanum* UTEX 485 when exposed to the aqueous solution of $\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$ at certain (25–100 °C) temperatures produces cubic Au NPs. However, changing the substrate to AuCl_4^- and lowering the exposure time, while raising the temperature (200 °C) drives the cyanobacterium to produce octahedral Au nanoplates (Lengke et al. 2006). In this particular study the NPs were observed to be formed at the cell wall.

Plants

Plants are also capable of producing a variety of NPs. The precise mechanism and intermediate steps of NP production in plants is not yet known but it is most likely a biomineralization process in which the substrate is transported from the roots to different plant organs, and after reduction the substrate molecules are deposited as NPs (Haverkamp and Marshall 2009; Makarov et al. 2014). *Brassica juncea*, *Festuca rubra* and *Medicago sativa* were all observed to intracellularly produce Ag NPs after the roots were exposed to an aqueous solution of AgNO_3 for 24 hours (Marchiol et al. 2014). Another approach of NP synthesis is using plant biomass. Incubating the biomass of *Avena sativa* with the aqueous solution of Au(III) ions resulted in the intracellular formation of Au NPs. This particular study concluded that oat biomass has the ability to take up and reduce Au(III) ions to Au(0) (Armendariz et al. 2004). Plant based extracellular NP formation is also possible. It has been reported that the incubation of *Azadirachta indica* leaf extracts with the aqueous solutions of AgNO_3 and HAuCl_4 yields polydisperse Au, Ag and Au-Ag bimetallic NPs (Shankar et al. 2004). Similarly, various shaped Au NPs are formed when the flower extract of *Cassia auriculata*, acting as a reducing agent, is incubated with the aqueous solution of HAuCl_4 (Dhayananthprabhu et al. 2013).

The last step in producing NPs is *functionalization*. This is the customization process in which NPs are bestowed with properties, beyond the ones that come with a certain material composition, to fit a specific or a set of specific functions. In practical terms functionalization is the attachment of specific linkers and ligands, application of specific coatings or the loading of NPs with certain biomolecules (Fig. 5).

Application of NPs for biosensing

One of the major fields of NP application is biosensing. This entails the detection of various molecules, cellular functions, pathogens and analytes. A biosensor is comprised of at least two elements: a biological recognition part, which actually interacts with the substrate and a signal transducer part, the role of which is to give off a measurable signal at the time of interaction. The average size of NPs is not much different from average sized biomolecules. This feature, together with their material-derived, specific properties (e.g., magnetism, semiconductivity, specific fluorescence) makes NPs a very sensitive tool in detecting molecules. The large surface area of NPs is an additional benefit through which they are able to interact with substrates. This also allows the application of various coatings and numerous ligands that may enhance

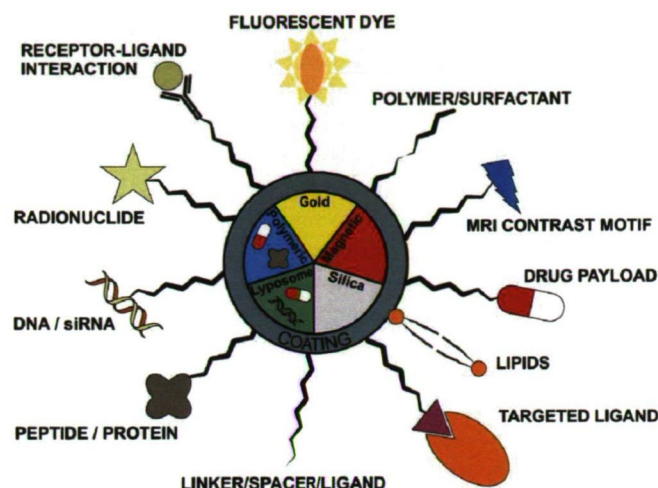


Figure 5. Functionalization of nanoparticles. Examples for ligands that may be attached to nanoparticles in order to render them suitable for a specific purpose.

the specificity of the particles and/or provide proper signal transducers.

Colorimetry

A colorimetric test involves a stable system to which the addition of a specific substrate modifies its stability, which alters the quality of the reflected light. In the case of a Pb biosensor the stable system is formed by Au NP aggregates, which are functionalized with oligonucleotides. The aggregate is formed via a DNAzyme that is composed of a catalytic and a substrate strand and binds to the functionalized Au NPs either in a head to tail or tail to tail fashion. The substrate strand is specifically cleaved in the presence of Pb^{2+} and upon disassembly the system changes color, from blue to red (Liu and Lu 2005). The classic example of a colorimetric assay builds on the oligonucleotide-dependent aggregation of functionalized Au NPs (Fig. 6). In this setup Au NPs are functionalized with single stranded DNA. Oligonucleotides with a specific sequence will act as a linker between NPs, and in their presence aggregates will form. Upon the formation of aggregates a red to purple color shift takes place (Elghanian et al. 1997).

Fluorescence based sensing

One of the inherent properties of metallic NPs is their energy quenching ability, which proved to be an excellent tool in NP-based biosensor engineering. Fluorescence sensing takes full advantage of this in producing Au NPs which are functionalized with fluorescently labeled oligonucleotides (Fig. 7). In its initial position, due the hairpin conformation of the

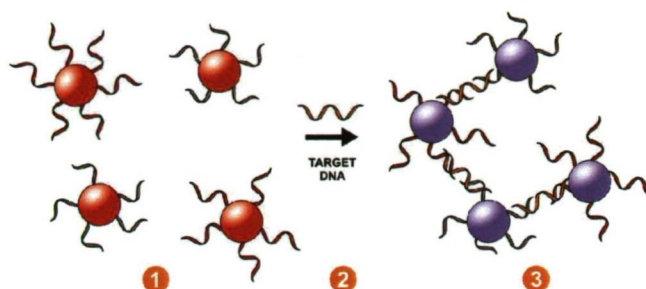


Figure 6. Colorimetric sensing. (1) Nanoparticles functionalized with single stranded DNA, complementary to the target DNA. (2) In the presence of the target DNA strand, the functionalized NPs aggregate. (3) A red to blue shift occurs.

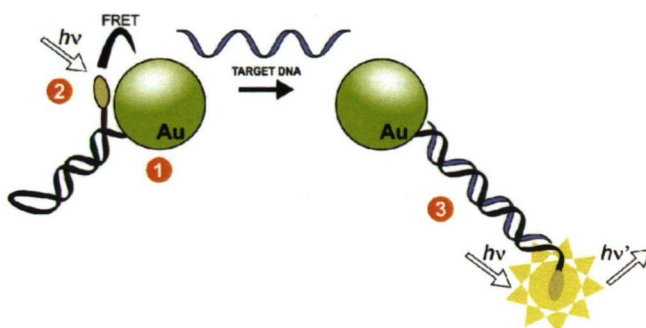


Figure 7. Fluorescence sensing. (1) Gold nanoparticles are functionalized with hairpin DNA strands, to which a fluorescent dye is attached. (2) The fluorescent dye is adsorbed to the gold nanoparticle, thus its fluorescence is quenched. (3) Upon binding the target DNA, the hairpin conformation is straightened out and due to the gained distance from the nanoparticle, the dye emits fluorescence.

single stranded oligonucleotides, the fluorophores adhere to the surface of the Au NP, and this results in fluorescence quenching due to Förster resonance energy transfer (FRET) towards the Au NP. In the presence of a complementary, target DNA strand the hairpin oligonucleotide will hybridize with it, the constrained conformation opens up and the fluorophore is separated from the particle surface resulting in high fluorescence yield providing proof for the presence of the target DNA (Maxwell et al. 2002). The same principle of fluorescence quenching is used to detect proteins, cells and pathogens. For the latter assays Au NPs are functionalized with ligands that bind various fluorophore agents, thus quenching their fluorescence. Owing to the specific design of the ligands, the target substrate, be it a protein or a cell surface moiety, will compete with the fluorophores in binding to the NP. As a consequence fluorescence quenching is removed leading to a fluorescence signal. These tests are also commonly known as chemical nose assays (Miranda et al. 2010).

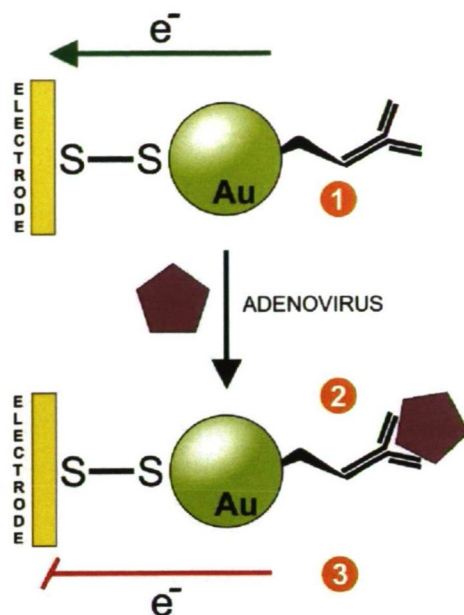


Figure 8. Electrochemical sensing. (1) Gold nanoparticles, with ligands that bind antibodies, are immobilized to an electrode surface. (2) The adenovirus specifically binds to the antibody. (3) The bound viruses create electrical resistance, which hinders the electron transport through the electrode.

Electrochemical detection systems

Due to the conductivity enhancing properties of metallic NPs they are quite popular in electrochemical detection systems. These assays usually involve NPs with detector molecules (e.g., antibody, enzyme) and an electrode surface. The actual detection measurements are carried out using voltammetry. In an interesting and recyclable virus detection assay Au NPs were attached to an Au electrode surface via thiol groups. Following this step the NPs were functionalized with a type of ligand that readily binds a specific antibody, which is able to detect a particular adenovirus (Fig. 8). Due to a desorption technique the aforementioned layers can be removed from and reassembled onto the electrode surface (Lin et al. 2015). Another assay is able to sensitively detect the human pathogen *Staphylococcus aureus* by creating a magnetic bead-pathogen-Ag NP sandwich. Ag^+ ions will dissolve in the buffer of the setup in a directly proportional manner to the pathogen's concentration. The ions are then transferred to an electrode surface and measured via differential pulse anodic stripping voltammetry (Abbaspour et al. 2015).

DNA and protein detection

An interesting biosensor aimed at specifically detecting DNA or proteins is the bio-barcode method. This technique requires

that complementary DNA strands or antibodies (for target DNA or target protein, respectively) are immobilized to a surface or to a NP (e.g., magnetic NP, for easy manipulation). During the next step, in the presence of target DNA or protein, the immobilized DNA strands or antibodies are hybridized with NPs (usually Au NPs), that are functionalized with bio-barcode DNA. After the immobilized DNA strands or antibodies fished out the corresponding bio-barcode NPs, the latter are dehybridized from the former and their bio-barcode DNA strands are eluted through washing and heating steps. At this point the signal of the target DNA or protein is highly amplified in the form of the released bio-barcode DNA. In a last step the bio-barcode DNA is detected in a chip-based microarray, where they are hybridized to immobilized oligonucleotides and NPs that allow for the visualization of the hybridization event (Fig. 9).

Using this technique the HIV-1 p24 antigen is readily detectable at an order of magnitude lower level than in the case of ELISA, and even more importantly the antigen is detectable at an earlier phase of infection (Tang 2010).

SERS-based sensing

Surface-enhanced Raman scattering (SERS) is another technique that takes advantage of certain features of NPs. Raman spectroscopy gives spectral information of molecular vibrations in mid-infrared and near-infrared (NIR) spectra. However, the infrared radiation or the Raman signals can be significantly enhanced by scattering. For this purpose the relatively large surface of NPs can be engineered rough enough to achieve proper scattering (Kim and Shin 2011). Generally this biosensing method entails metallic NPs (e.g., Au, Ag, Cu) being in contact with the target molecule or entity. A good example for the sensitivity and applicability of this biosensor is the detection of foodborne pathogens. One particular method uses Ag NP-encapsulated biopolymers as substrate for the bacteria and through this *Salmonella Typhimurium*, *Escherichia coli*, *S. aureus* and *Listeria innocua* could be detected with a high selectivity (97-99%) (Sundaram et al. 2013). Another approach is to have the target pathogen internalize the scatter-enhancing Ag NPs. This additional step enhanced the detection sensitivity of SERS and waterborne bacteria (*Staphylococcus epidermidis*, *Listeria monocytogenes*, *Enterococcus faecalis*) could be differentiated down to single cells (Fan et al. 2011).

Utilization of NPs as delivery systems

Due to their small size and our ability to functionalize them with various ligands, NPs are effective tools to transport an array of molecules to specific sites. This approach can be

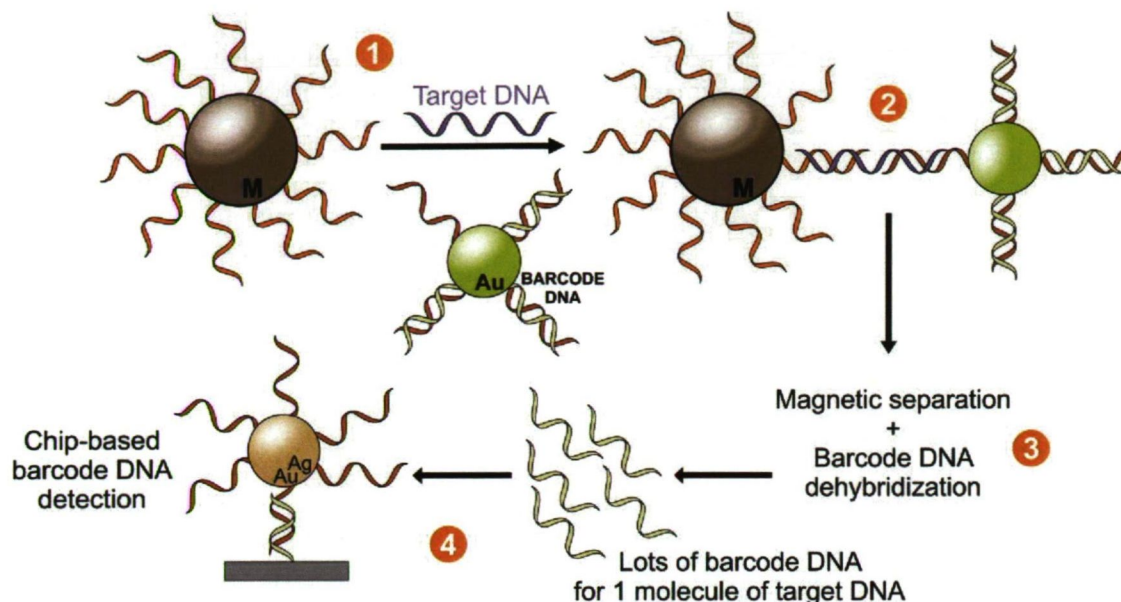


Figure 9. Bio-barcode method. (1) Complementary DNA strands are immobilized to magnetic (M) NPs. (2) Through the target DNA acting as a linker, M NPs are hybridized to bio-barcoded gold (Au) NPs. (3) By applying a magnetic field the hybridized setup is collected. (4) Barcode DNA is eluted and used for visual chip-based detection of the target DNA signal.

used in research (for delivery of DNA, RNA, proteins and other molecules *in vitro* and *in vivo*) as well as in clinical applications (for *in vivo* delivery of drug molecules to certain tissues and even to specific cells). Once the NPs enter the *in vitro* or *in vivo* system the release of their payload might be targeted and triggered in a passive or an active manner, or via a combination of the two.

Passive targeting

This approach relies mainly on the properties of the targeted tissues and cells. Additionally the surface charge and the optional coating of the NP might play a role. The principle of the process is best explained through the Enhanced Permeability and Retention (EPR) effect of tumor tissues. Two of the important aspects in which tumor tissues differ from normal tissues are that their vasculature system is more permeable and their lymphatic drainage is poor. Consequently NPs of various sizes can leave tumor capillaries and readily accumulate in the interstitial space (Torchilin 2011). As a result tumor tissues retain more NPs than normal tissues. The EPR effect is even more effective if the NPs stay in circulation as long as possible, for example due to polymer coatings (Torchilin 2006). The most widely used of these coatings is polyethylene glycol (PEG), a hydrophilic polymer, which successfully retards opsonization and thus delays internalization of NPs by macrophages.

Active targeting

This approach relies on certain functionalized and engineered features of NPs. One main branch of this category is receptor-mediated targeting, in which NPs are functionalized with ligands for specific recognition of receptors and antigens (Torchilin 2006). The other main branch is stimuli-sensitive targeting, in which NPs are engineered so that they may be triggered by local environmental changes, like pH, temperature, enzyme activity, ultrasound, magnetism, etc. (Arias 2011).

Antibody-based targeting

Within the receptor-mediated, active targeting category the most straightforward solution is the use of monoclonal antibodies against specific tumor antigens. A variety of monoclonal antibodies may be attached directly to liposomes, or to the PEG coating of liposomes (Imai and Takaoka 2006). A great example for what these liposome NPs are capable of is the following: PEG-coated liposomes were functionalized with an antibody against a transferrin receptor, so that the NP can cross rat blood brain barrier, and with another antibody against the insulin receptor to target specific cells within the brain. These particular NPs were loaded with a plasmid that encodes a short hairpin RNA sequence, which is able to silence a certain oncogenic gene (Zhang 2003). In connec-

tion to the latter study, it has been reported that transferrin receptors are highly expressed in lung cancer, breast cancer and lymphoma tissues (Dowlati et al. 1997). Thus *transferrin*, which is a β -glycoprotein that facilitates the transport of Fe^{3+} ions across the cell membrane, may be used to target specific tumor cells and it is particularly useful in overcoming the blood brain barrier.

Vitamin-based targeting

The most promising among the variety of vitamins with which NPs may be functionalized, is folate. It has been shown that folate receptors are absent in most normal tissues but even more importantly they are overexpressed in a variety of tumor tissues (Sudimack and Lee 2000).

Lectins are carbohydrate binding proteins that are also used as ligands for cancer-targeting NPs. There are two main advantages of using lectins: on the one hand they are slightly toxic and are able to induce apoptosis of malignant cells while activating the immune system (Udey et al. 1980), and on the other hand different types of lectins specifically bind to certain tumor tissues that express certain carbohydrate groups (Lavanya et al. 2014).

Peptide-based targeting is highly successful in targeting the vasculature of tumor tissues. Most applications rely on the RGD sequence (arginine-glycine-aspartic acid), present in a number of proteins, among which integrins are the most important as they are receptors for cell adhesion molecules. This carries the potential that RGD-functionalized NPs adhere to tumor cells with higher affinity, since these overexpress integrins (Pasqualini et al. 1997). One *in vitro* study revealed that the uptake of liposomes functionalized with peptides containing the RGD sequence was more efficient than the uptake of non-functionalized liposomes (Dubey et al. 2004). Peptide ligands are considered fairly advantageous, mainly because they are relatively short, hence they possess high stability and they are easily and rapidly synthesized.

Aptamer-based targeting

Aptamers are oligonucleotides (either DNA or RNA) which by forming three dimensional structures are able to bind and interact with antigens (Cech 2004). Aptamers are an effective tool even without a NP loaded with drugs, and they are able to target various molecules (*e.g.*, growth factors, hormones, antibodies), and even organisms (Pestourie et al. 2005). However, due to the wide selection of binding targets aptamers have the potential to be particularly useful as NP conjugates, and thus for drug delivery. In one example polymer NPs were functionalized with an aptamer that targeted a prostate specific membrane antigen, which is upregulated in prostate cancer. The study used rhodamine-labeled dextran as a drug payload, which was successfully delivered to the target tissue

(Farokhzad et al. 2004).

pH-responsive targeting is based on the phenomenon that liposomes and polymeric NPs that are stable at physiological pH values tend to dissociate or degrade at different pH levels, upon which their drug payload gets released. The principle behind this process is that most of these NPs are engineered with phosphatidylethanolamine (PE) in their membranes, which changes from a lamellar to an inverted micelles form at low pH, and as a result fusion of liposomal and endosomal membranes is made possible (Karanth and Murthy 2007). Since due to their different metabolic characteristics tumor interstitial tissues have lower pH levels than the surrounding tissues (Tannock and Rotin 1989), this slightly more acidic environment may provide a specific triggering signal for the right type of NPs. It is not only cancer therapy that makes good use of this method. In basic research, when for example antisense oligonucleotides need to be delivered and triggered in a specific manner, pH-responsive liposomes are also a smart choice (Fattal et al. 2004). Another approach to this technique is to have the proper NPs target and fuse to intracellular compartments with low pH, for example lysosomes (Simoes et al. 2004).

Relatively high temperatures can also be used for triggering a payload release in cancer therapy, but local hyperthermia has other therapeutic effects as well. One important effect is of course the drug release, which is achieved by using thermo sensitive NPs, loaded with drugs, that are made of polymers or hydrogels, with specific thermo sensitive ingredients in their structure. Poly(N-isopropylacrylamide) (PNIPAAm) is the most popular of these ingredients, that, in response to a certain temperature change, undergoes a sharp phase transition in water with which its hydrophilicity temporarily turns into hydrophobicity (Gil and Hudson 2004).

Besides the drug release, it has also been shown that local hyperthermia in itself is toxic to tumor tissue (Jones et al. 2006), plus, increased temperatures further enhance its already high extravasation tendency, thus facilitating further NP delivery to tumor tissues (Kong et al. 2000). Additionally, local hyperthermia is quite useful to chemosensitize cancer cells (Urano et al. 1999), an effect for which Au NPs, superparamagnetic iron oxide NPs (SPIONs) and even carbon nanotubes (CNTs) are used (Chatterjee et al. 2011). Applying an alternating magnetic field to well localized SPIONs or electromagnetic radiation of certain wavelengths to Au NPs or CNTs will result in selective heating of target tissues (Urano et al. 1999).

To transfer energy to Au NPs and CNTs generally NIR light is used, mostly for its good penetration properties, but also because it can generate considerable heat by itself. The latter property of NIR radiation makes it an optimal candidate to be used as a drug delivery trigger, as an agent in the production of local hyperthermia and even bioimaging, thus potentially combining several kinds of therapies and diagnos-

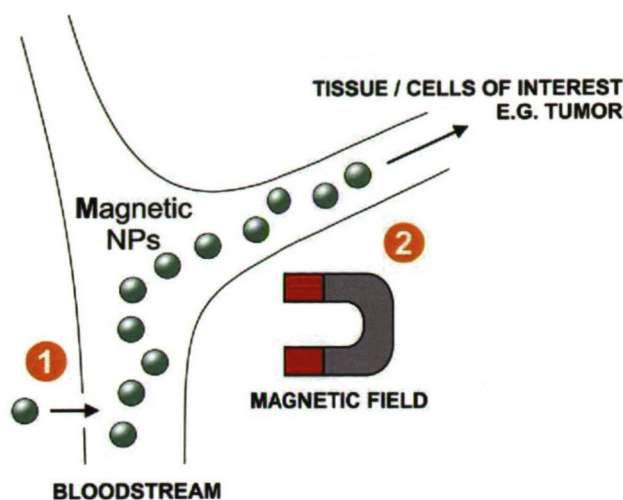


Figure 10. Magnetic NPs as delivery systems. (1) Magnetic (M) nanoparticles (NPs) are introduced into the vasculatory system. (2) Through the application of a magnetic field M NPs may be externally modulated (guided, aggregated, heated, etc.)

tic processes, giving rise to the term theranostics.

Magnetic NPs, by producing a strong enough magnetic field, are easily guided and concentrated to certain locations within an organism (Fig. 10). Additionally, the technique has the potential to keep the respective NPs in place for a certain period of time during which another stimuli-based trigger may be applied, or the particles may heat the surrounding tissue via transferring energy from a magnetic field, thus facilitating cancer therapy. Since metallic NPs are inferior to polymer and liposome NPs in terms of payload capacity, the optimal design to make good use of magnetic properties is either a particle with a magnetic core and polymer coating (Arias et al. 2008) or a polymeric carrier with magnetic NPs in its structure (Ciofani et al. 2009).

Ultrasound provides another non-invasive method for targeting and triggering NPs. The effect of this method is multifaceted, but it is mainly based on the principle of enhancing permeability: it increases vasculature and cell permeability (Lammertink et al. 2014); it also generates heat which further enhances the permeability of surrounding biomembranes and presents a stimuli to thermal-sensitive NPs (Ta et al. 2014). These effects, of course, facilitate the extravasation and cell-uptake of NPs. Ultrasounds, depending on their intensity, can penetrate tissues while focused to certain locations and are able to disrupt the structure of polymer NPs and therefore enhance their payload release (Phenix et al. 2014).

Enzymes are among the various biomolecules, which are overexpressed in tumor tissues, and may potentially be used as triggers for specific drug delivery. The lipid hydrolyzing

phospholipase A2 (PA2) family of enzymes is overexpressed and released into the interstitial space of several types of cancers (e.g., breast, stomach, pancreas) (Abe et al. 1997). Hence by engineering suitable liposomes, the drug carried by these NPs will be efficiently released upon contact with elevated PA2 levels (Jensen et al. 2004).

Use of NPs in imaging

NPs have the potential to revolutionize research techniques and clinical therapy, however, their potential side effects should also be considered. Using NPs for *in vitro* imaging experiments does not present any problems, but using them *in vivo* and/or in theranostic applications, unveils some major concerns. For example in traditional clinical applications imaging dyes or particles are not supposed to have physiological effects and it is an advantage if they are excreted in a short time, in a controlled manner. In contrast to this expectation some NPs might accumulate in an uncontrolled fashion in certain tissues, thus increasing their chances for unwanted interactions. Additionally, it is still not precisely clear, and hence is a major concern, how and in what time frame organisms can eliminate NPs of certain sizes and materials. Naturally a swift biodegradation and elimination is preferred to a prolonged retention, in all cases. In spite of these concerns using NPs in various imaging techniques offer significant advantages.

Optical imaging

Using optical methods for imaging is a non-invasive and cost-effective approach. It has of course its limitations, among which, when working with *in vivo* systems, its relatively small penetration depth is emphasized. A popular solution to this problem is the use of NIR spectrum as excitation light in optical fluorescence imaging, since this radiation is able to penetrate deeper into a given sample tissue. Several fluorophores have been developed to be used with NIR light, however, the use of NPs for this purpose has some major advantages. The most popular NPs used in optical imaging are quantum dots (QDs). These are NPs made from semiconductor materials with diameters small enough to exhibit quantum mechanical properties. Small enough means similar or smaller than the size of the semiconductor's exciton Bohr radius, which is the average distance between the electron in the conduction band and the hole it leaves behind in the valence band. In such a small particle excitons are confined in all three spatial dimensions, the structure of energy levels is dependent on the size of QD. The conduction and valence band are quantized and the band gap energy increases by decreasing the size of QD.

As a result of it more energy is needed to excite a dot, and apparently more energy is emitted when the electron returns to its ground state, resulting in shift in the color of the emitted light from red to blue by decreasing the dot size. Due to this quantum phenomenon the color of fluorescence emitted by the dots can be tuned only by changing the size of QDs.

For imaging applications either plain QDs (e.g., CdSe, CdTe, GaAs) or QDs with certain dyes attached to them (Zhu Yian et al. 2013) may be used. In contrast to fluorophores QDs are generally more stable photochemically (are exempt from photobleaching) and metabolically (Loginova et al. 2012), are brighter and have a narrower, tunable, symmetrical emission spectrum (Bruchez et al. 1998). Metabolic stability is of course a double edged sword because a lingering amount of QDs in an *in vivo* system might have toxic effects. However, this aspect might be modulated through particle size and various coatings (Choi et al. 2007). Certain coatings are also necessary to counteract the hydrophobicity of QDs and facilitate their application in aqueous systems (Michalet 2005).

QDs owe their valuable optical properties to quantum confinement effects, meaning that merely depending on their size their properties can vary. One important consequence of this feature is that in response to the same excitation light, QDs of the same material but with different sizes respond with significantly different emission spectra (Bentolila et al. 2005). Since generally QD sizes range from 2-10 nm, they can be used to image subcellular compartments as small as actin fibers (Bruchez et al. 1998) and even cell signaling pathways (Lidke et al. 2004).

Another way to bypass the depth penetration limitations of optical imaging through the use of QDs is the functionalized, self-illuminating QD. One elegant example for this method involves QDs (core/shell structured CdSe/ CdS) that are functionalized with peptide linker sequences, which on the other hand bind Au NPs, that are quenching the strong luminescence of the QDs. Upon encountering the right enzyme, the peptide linkers are degraded, the Au NPs are released and the QDs give off measurable luminescence (Fig. 11). The elegance of the technique derives from the fact that it can be customized by adjusting the peptide linker sequence to match the functions of particular enzymes (Chang et al. 2005).

Magnetic resonance imaging

Certain NPs, due to their dense materials (e.g., QDs: Ga, Cd, Pb) are ideal contrast agents for magnetic resonance imaging (MRI). MRI is a noninvasive method with an efficient depth penetration and high spatial resolution (10-100 μm) that measures the relaxation of hydrogen protons in different microenvironments. In biomedical applications the role of contrast agents is to provide efficient distinction between tissues and fluids. Typical NPs for this task are gadolinium (Gd)-loaded

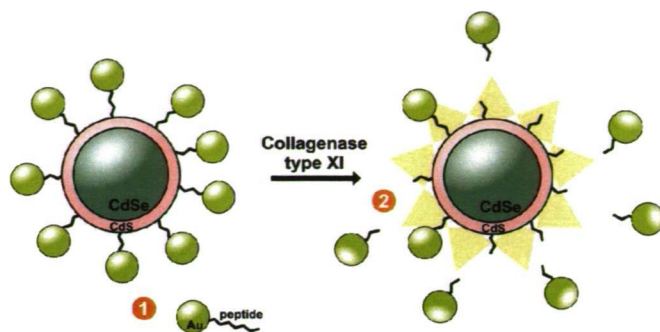


Figure 11. Quantum dots (QDs) in optical imaging. (1) QDs (core/shell structured CdSe/ CdS) are functionalized with peptide linker sequences, that bind gold nanoparticles (Au NPs). QD fluorescence is quenched by Au NPs. (2) In the presence of a specific enzyme the linkers are disrupted. As Au NPs move away from the QDs, fluorescence commences.

polymers (Xu et al. 2007), SPIONs and manganese-based NPs (Felton et al. 2014). Functionalizing the NPs makes it possible to specifically target particular tissues and cells. For example SPIONs functionalized with single chain Fv antibody fragments were successfully utilized to target cancer cells that overexpressed a carcinoembryonic antigen (Vigor et al. 2010). The serious drawback of these excellent contrast agents are however, that both, Gd-based and magnetic NPs show signs of toxicity (Chang et al. 2013).

A unique feature of NPs is that via clever engineering and multivalent attachments a single kind of NP might be applied in several different techniques. For example optical imaging and MRI might be combined via specific NPs (Zhou et al. 2010). Taking it a step further and adding a drug delivery event to the imaging processes, a promising theranostic tool has been reported recently. A cell nucleus targeting mesoporous silica NP has been developed that is able to transport the radiosensitizing drug Mitomycin C directly to the cancer cell's DNA. In this researchers combined the previously mentioned dual-modality imaging properties with nuclear-targeting drug delivery NPs (Fan et al. 2015).

Computer tomography

CT is the 3D application of conventional X-ray analysis. The method gathers signals via detecting the X-ray attenuation of various tissues. Contrast agents enhance this effect thus giving clearer signals. The classic contrast agents for CT scans are iodine based products, which are known for their toxicity on the kidneys (Perrin et al. 2012). To reduce their toxicity and enhance their circulation time liposome NPs are applied as carriers (Elrod et al. 2009).

Utilization of NPs to increase enzyme stability

Immobilizing enzymes to NPs produces efficient biosensors but that is not the only practical advantage that the process offers. Developments in production and application of NPs led to the realization that enzymes immobilized to NPs are active over a wider range of pH than their counterparts in solution, with an increased thermal stability. Besides these beneficial points industrial enzymatic applications gain advantages through the relatively low cost of the setup, the ease of handling it, the high surface to volume ratio of NPs that hosts more molecules than a 2D surface, and the efficient recuperation of the enzymes from the reaction mixture. In the immobilization process enzymes should not suffer denaturation and are required to retain their biocatalytic activity over a certain period of time. Matching these requirements various types of NPs have already successfully found application.

Immobilization with gold NPs

With excellent biocompatibility Au NPs are an obvious choice for immobilization. On the one hand they may be functionalized with thiol or carboxylic groups that can bind peptides and proteins (Lin et al. 2015), on the other hand they readily interact with the cysteine groups of proteins (Jeynes et al. 2013), although direct protein-NP interaction may alter the proteins' functional structure (Lundqvist et al. 2008). Furthermore, the conjugation of proteins on colloidal gold NPs might also result from electrostatic interactions between negatively charged citrate on surfaces of Au NPs and positively charged groups of the proteins (Zharov et al. 2006). In one study glucose oxidase has been successfully conjugated to Au NPs, which significantly enhanced the enzyme's thermal stability (Li et al. 2007). The same enzyme immobilized to thiolated Au NPs produced a response time of 30 seconds and a shelf life of more than 6 months (Pandey et al. 2007).

Immobilization with magnetic NPs

Among major advantages magnetic NPs offer in enzyme immobilization the most significant is the option to externally modulate the NPs' properties and location. In practice, in most cases, this is the easy separation and recovery of enzymatic complexes from the reaction mixtures (Ren et al. 2011). The process typically uses magnetic NP-silica composites because of the more favorable biocompatibility, although for example the direct binding of cholesterol oxidase to iron oxide NPs brought significant benefits to the enzyme activity: enhanced tolerance to pH, temperature and substrate concentration (Kouassi et al. 2005). In another example conjugation of carboxypeptidase from *Sulfolobus solfataricus* to iron oxide

NPs, via Ni²⁺/His-tag, resulted in the enhanced stability of the enzyme at room temperature and in organic solvents at high temperature (Sommaruga et al. 2014). Similarly, lipase from *Candida rugosa* has been immobilized onto polydopamine coated magnetic iron oxide NPs and as a consequence its pH and thermal stability was significantly increased (Ren et al. 2011).

Immobilization with silica NPs

Besides using it as coating material for magnetic NPs, silica represents an individual group of NPs used for immobilizing enzymes. The simplest way of immobilizing an enzyme to a NP is via adsorption. In a study α -chymotrypsin and lipase were immobilized with this method to mesoporous silica NPs, after which the enzymes formed crosslinked aggregates. This setup ensured that the immobilized enzymes had significantly elevated stability, compared to the free form (Kim et al. 2007). An exceptionally interesting scenario is in which a team managed to not only immobilize glutamate dehydrogenase and lactate dehydrogenase to silica NPs but also succeeded in immobilizing their cofactor NAD(H), thus giving way for multi-step enzyme activities and also *in situ* cofactor regeneration (Liu et al. 2009).

Nanotoxicity

Special physicochemical properties of NPs differ substantially from the bulk materials of the same composition. These qualities, and not yet fully understood interactions of NPs with biological systems and with the environment, may lead to potentially toxic processes. The biological impacts and kinetic properties of NPs are dependent on size, chemical composition, surface structure, solubility, shape, aggregation, and also on the physical and chemical parameters of the environment, like temperature, pH, irradiation, absence and presence of oxygen, electric or magnetic field etc. (Bennet and Kim 2014; Nel et al. 2006). The interaction of these features may modify various processes (e.g., cellular uptake, protein and DNA binding, translocation from entry to target site, eventual production of reactive oxygen species (ROS)) and carries the possibility of tissue injury (Nel et al. 2006; Choi and Wang 2011; von Moos and Slaveykova 2014). Potential entry routes of NPs into the human organism include the gastrointestinal tract, skin, lung, eye, nostrils, lips, mucus membrane, intravenous administrations for diagnostic and therapeutic purposes (Nel et al. 2006; Yah et al. 2012).

Due to increasing production of engineered NPs concerns about the environmental risks are growing, however, currently little is known about the exact NP concentration in the environment. Using probabilistic material-flow modeling, based

on the newest production volume data, the concentration of NPs in the air, soil, surface water, sediment etc. can be calculated (Garner and Keller 2014; Gottschalk et al. 2009; Sun et al. 2014). It was shown that production volume and inertness of compounds are the crucial factors determining final concentrations (Sun et al. 2014). Results of the modeling showed that risks to aquatic organisms may emanate from Ag NP, TiO₂ NPs and ZnO NPs in sewage treatment effluents and from Ag NP in surface waters (Gottschalk et al. 2009).

Toxicity of SiO₂ NPs

Although the use of engineered silica NPs is steadily increasing and the production volume of silica NPs is the highest, information on their potential health risk is scarce, most likely because they are generally perceived as non-toxic. However, there are studies showing that even silica NPs can have adverse effects on biological systems. It was demonstrated that silica NPs inhibit the total oxygen uptake in activated sludge during wastewater treatment, which shows that aerobic respiration, essential to the biological oxidation of organic matter, is damaged by silica NPs (Sibag et al. 2015). In the same study it was shown that smaller (12 and 151 nm) silica NPs showed a higher inhibitory effect than larger (442 and 683 nm) ones, and also that silica NPs significantly altered the composition of microbial membrane lipids (Sibag et al. 2015). *In vivo* genotoxic effects were studied of four different nano-size forms of silica NPs in *Drosophila melanogaster*, and significant dose-dependent increases were found in the levels of primary DNA damages, but no genotoxic effects were obtained with microparticulated silicon dioxide (Demir et al. 2015). Silica NPs (average size of 62 nm) induced autophagy and autophagic cell death in human hepatocellular carcinoma (HepG2) cells triggered by ROS, suggesting that exposure to silica NPs could be a potentially hazardous factor for maintaining cellular homeostasis (Yu et al. 2014). Silica NPs with 10–20 nm diameter size also decreased the growth and chlorophyll content of *Scenedesmus obliquus* when they were added to the cell culture at 200 mg/l concentration (Wei et al. 2010).

Toxicity of Ag NPs

The toxicity of Ag NPs on various organisms was reviewed in several publications dealing mainly with the antimicrobial effect of Ag NPs, but also with the potential hazard on human health and the environment. (Lara et al. 2011; Maillard and Hartemann 2012; Marambio-Jones and Hoek 2010; Mijnen-donckx et al. 2013; Rai et al. 2009; Reidy et al. 2013). It was found that the pathways involved in bacterial responses (*E. coli*) to Ag NPs are highly dependent on physicochemical properties of the NPs, particularly surface characteristics and toxicity mechanisms of Ag NPs are different from ionic silver

(Ivask et al. 2014). Quantitative proteomics studies on human colon cancer cell lines indicated that some cellular responses triggered by Ag NPs are driven by the size of NPs (Verano-Braga et al. 2014). The 100 nm NPs exerted indirect effects via serine/threonine kinase (PAK), mitogen-activated protein kinase (MAPK), and phosphatase 2A pathways, while the 20 nm NPs induced indirect effects on cellular stress, including generation of ROS, protein carbonylation and up-regulation of proteins involved in SUMOylation (Verano-Braga et al. 2014). The toxicity of Ag NPs may further increase due to slow dissolution of silver ions from the surface of NPs. The rate and degree of dissolution depends on the functionalization and also on the temperature, but in some cases NPs can release up to 90% of their weight in form of silver ions (Kittler et al. 2010). In addition to dissolution, aggregation and ROS generation can also occur to Ag NPs in an aqueous environment. These processes are dependent on NP surface coatings and on irradiation conditions (Li et al. 2013). It was found that UV-A (365 nm) irradiation (compared to UV-C 254 nm and xenon lamp) resulted in the highest released silver ion concentration and generated superoxide and hydroxyl radicals in bare Ag NPs (Li et al. 2013). As it is known from radiometer measurements 10% irradiance depth for the UV-A radiation varies between few meters to few tens of meters in various marine environments (Tedetti and Sempere 2006), so solar UV radiation can have a significant impact on the toxicity effect of Ag NPs in natural waters. The inhibitory effect of Ag NPs on growth and on photosynthetic activity was studied in the cells of various algae, diatoms and cyanobacteria (Burchardt et al. 2012; Dewez and Oukarroum 2012; Matorin et al. 2014; Navarro et al. 2008; Oukarroum et al. 2012) furthermore the toxicity of silver was investigated by complex transcriptomic and proteomic analysis in the green alga *Chlamydomonas reinhardtii* (Pillai et al. 2014). The toxic effect of Ag NP on photosynthetic activity of the cyanobacterium *Synechocystis* PCC 6803 can be observed by the detection of effective quantum yield of photosystem II (Fig. 12). Chlorophyll fluorescence measurements achieved by application of saturating light pulses on top of a continuous illumination with various light intensities clearly showed that shortly after the addition of Ag NPs, at concentrations of 1 ppm and above, growth inhibition of these cyanobacterial cells occurs (Fig. 12).

Toxicity of TiO₂ NPs

The estimated annual production volume of TiO₂ NPs is around 3000 tons, the second highest volume among engineered nanomaterials (Bondarenko et al. 2013). TiO₂ NPs are used in sunscreens, medical implants, drug delivery, sensors, lithium ion batteries, wastewater disinfection etc. (Tilly et al. 2014). Modeling calculations indicated that the TiO₂ NPs in sewage treatment effluents may represent a risk to aquatic

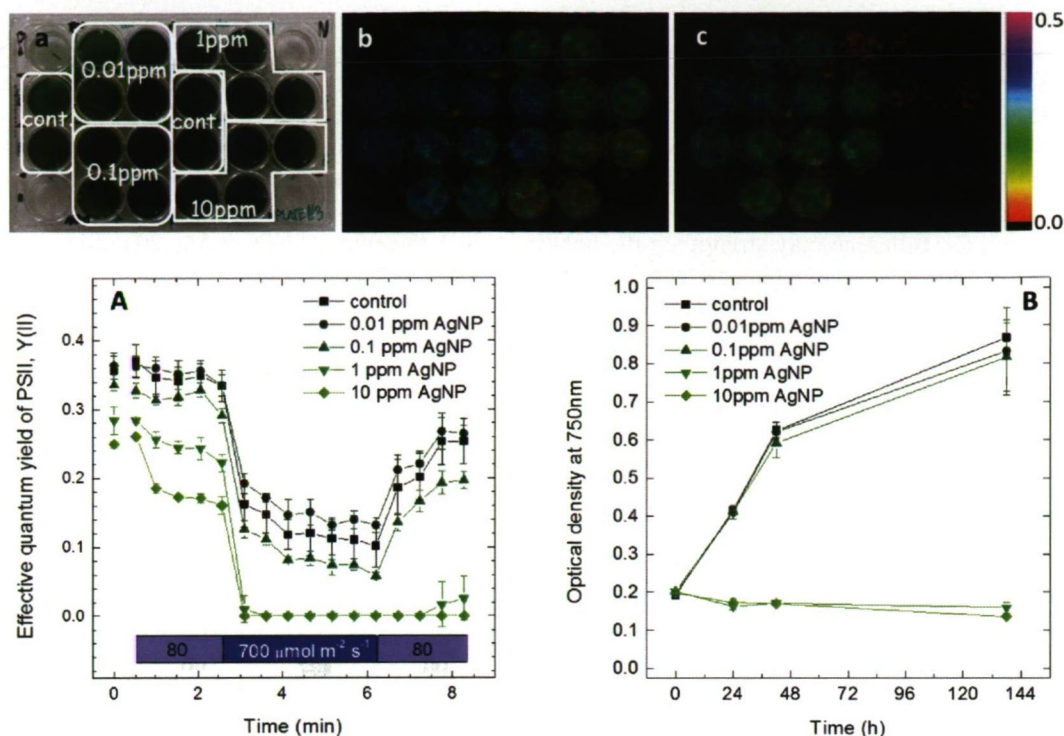


Figure 12. Effect of AgNPs on Chl fluorescence quenching. *Synechocystis* PCC 6803 cells were treated with AgNPs of 2 nm average size. The high intensity blue (450 nm) light induced quenching was detected by imaging of Chl fluorescence from the 24 well plates after incubating the cells for 90 min with indicated concentrations of AgNPs (image a: RGB photo) at growth conditions. False color coded images of the effective quantum yield of photosystem II ($Y(II) = (F_m' - F) / F_m'$ where F and F_m' are the chlorophyll fluorescence yields measured before and during a saturating pulse in an illuminated sample) obtained at the end of the first low intensity illumination period (image b) and at the end of the second low intensity illumination period (image c) are also shown. The growth of the cells was also monitored in the 24 well plates (Panel B)

organisms (Gottschalk et al. 2009). TiO_2 NP exposure of nitrogen-fixing cyanobacteria *Anabaena variabilis* led to observable alterations in various intracellular structures and induced a series of stress responses, including production of ROS, increase in the abundance of membrane crystalline inclusions, disruption of thylakoid and plasma membranes. Furthermore, cell surface morphology and mechanical properties were modified as well (Cherchi et al. 2011). Oxidative stress mediated by photoactive TiO_2 is the likely mechanism of its toxicity. Even relatively low levels of UV radiation, comparable with those found in nature, can induce toxicity of TiO_2 NPs to marine phytoplankton (Miller et al. 2012). Using the cyanobacterium *Synechocystis* PCC 6803 it was shown that TiO_2 NPs can trigger direct (cell killing) and indirect effects (cell sedimentation), and that these toxic effects are increased with NP concentrations, with UVA radiation, and in the absence of extracellular polymeric substances, (Planchon et al. 2013). Metabolomic analysis on the toxicological effects of TiO_2 NPs in mouse fibroblast cells revealed perturbations in the metabolism of certain amino acids indicating that TiO_2 NPs influence the cellular metabolic environment (Bo et al.

2014). Standard short-term (5 days) inhalation study on rats with aerosols of various NPs placed TiO_2 NPs into the grade of higher toxic potency (Landsiedel et al. 2014).

Toxicity of carbon NPs

Carbon-based nanomaterials, such as two-dimensional graphene nanosheets, one-dimensional CNTs or zero-dimensional fullerenes are widespread in different areas of nanotechnology. Graphene nanosheets can induce the degradation of the inner and outer cell membranes of *E. coli*. They also reduce cell viability and can penetrate into cell membranes and extract large amounts of phospholipids due to strong dispersion interactions between graphene and lipid molecules (Tu et al. 2013). Toxicological assessment of synthesized CNT-polymer hybrids as potential materials for membranes used in water treatment applications revealed that surface characteristics play a major role in the biological response of functionalized CNTs (Koromilas et al. 2014). The importance of surface modification of multiwall CNTs (MWCNTs) was also compared regarding cytotoxicity of raw MWCNTs and

MWCNTs functionalized with carboxylation (MWCNTs-COOH) or polyethylene glycol (MWCNTs-PEG) in murine macrophages and was found that only raw MWCNTs and MWCNTs-COOH altered the oxidative potential of macrophages (Zhang et al. 2015). Fullerenes in pure unmodified form have not shown any adverse effect (Johnston et al. 2010), but *in vitro* toxicological studies showed that occupational co-exposure with C₆₀ fullerene may strengthen the health effects of organic industrial (acetophenone, benzaldehyde, benzyl alcohol, m-cresol and toluene) chemicals (Lehto et al. 2014).

Awareness of nanotoxicity

The exponentially increasing number of scientific publications dealing with the toxicity effect of NPs reflects the responsible attitude of the scientific community. The Organization for Economic Cooperation and Development (OECD) launched a programme in 2006 to ensure that the approaches for exposure and risk assessment regarding manufactured nanomaterials are of a high quality, science-based and internationally harmonized. In 2012 they specified a list of 13 representative engineered nanomaterials, which can support measurements, toxicology and risk assessment of nanomaterials (Oecd 2012): fullerenes (C₆₀), single-walled and multi-walled carbon nanotubes (SWCNTs and MWCNTs), silver and gold NPs, NPs of iron, titanium dioxide, aluminum oxide, cerium oxide, zinc oxide, silicon dioxide, dendrimers and nanoclays. Concern-driven integrated approaches are published on a risk assessment of manufactured nanomaterials highlighting important issues such as public, occupational and environmental exposure to NPs, human health and ecological effects of NPs, persistence, bioaccumulation, fate and distribution of NPs (Behra and Krug 2008; Oomen et al. 2014).

Use of NPs in agriculture

The use of nanotechnology for crop protection presents novel applications for agriculture. Agri-nanotechnology has opened a new way of plant transformation, improved plant disease resistance, and crop protection (Nair et al. 2010). Effects of various NPs on the growth and metabolic functions of plants, as well as their uptake efficiency vary among plant species. The size of NPs has a strong effect on their interactions with living cells, influencing uptake efficiency, internalization pathway selection, intracellular localization and cytotoxicity (Chen and Yada 2011). NPs taken up by plant roots are carried to shoots through vascular systems depending on the plant anatomy and composition, as well as on the shape and size of NPs (Ma et al. 2010). Other entry routes include leaves, on the surface of which accumulation of NPs cause

foliar heating that results in alterations of gas exchange due to stomatal obstruction, eventually inducing changes in various physiological and cellular functions (Monica and Cremonini 2009). Generally leaf surfaces absorb NPs via stomatal openings or bases of trichomes and are distributed to various tissues (Eichert et al. 2008; Fernandez and Eichert 2009). Once the NPs make it past the leaf surface plant cell walls block the entry of NPs of 5 to 20 nm in diameter (Fleischer et al. 1999) that could potentially target specific delivery of proteins, nucleotides and chemicals. However, engineered NPs could increase cell wall pore size, or induce new cell wall pores, which in turn may enhance NP uptake (Navarro et al. 2008) and NP delivery systems can reach the chloroplast and mitochondria. NPs of calcium phosphate, carbon materials, silica, gold magnetite, strontium phosphate etc. can be combined with chemical compounds to deliver genes into target cells with minimum cell damage.

Nanopesticides

Development of products that can deliver long term effective and smart targeted agrochemicals like fertilizers or pesticides, fabrication of sensors for on-field rapid detection of contaminants and for improving seed germination can be achieved by functionalization of NPs with organic and inorganic molecules (Dhillon et al. 2012). Pesticides and fertilizers in the form of NPs can be very effectively transported to targeted sites and tissues, and their controllable use reduces unwanted side effects (Nair et al. 2010). Processes such as nanoencapsulation show the benefit of more efficient use and safer handling of pesticides with less exposure to the environment that aims for better ecoprotection.

NPs can impact plants on physiological, biochemical and genetic levels, the scale of which depends on plant species, NP properties and their concentration (Monica and Cremonini 2009). NP intake could drastically affect plant biodiversity, where more sensitive species may be eliminated and growth, flowering and fructification of other species may be favored through the presence of NPs (Monica and Cremonini 2009). Nanosilica treatment exhibits germination enhancement in maize seeds, along with the increase of root-shoot length and the number of newly emerging lateral roots in comparison to control plants (Dhillon et al. 2012). Risk assessment should be carried out on interactions of NPs with plants, which are essential base components of all ecosystems (Ma et al. 2010).

NP- mediated genetic transformation in plants

Vehicles for nuclear transformation in plants include *Agrobacterium* mediation, microparticle bombardment, and electroporation. *Agrobacterium* mediated transformation is most extensively used because of its wide host range. The expensive microparticle bombardment technique delivers DNA into

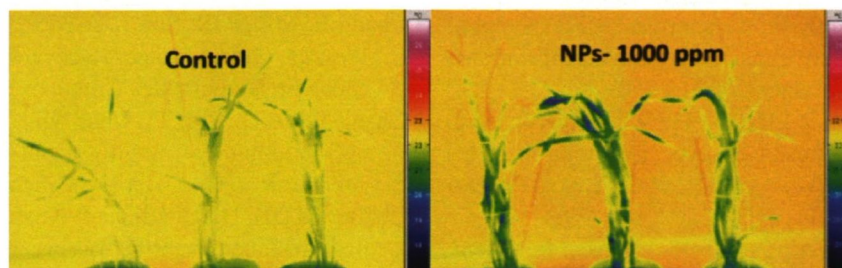


Figure 13. Thermal images of wheat seedlings (GK Élet cv.) grown under hydroponic conditions. Leaf temperature was measured by a thermal camera after 15 days post treatments of control Hoagland solution and nanoparticle dispersion (SiO_2 NPs, 1000 ppm). The darker color indicates cooler leaf temperature.

the nucleus, but may damage mitochondria, and it has a high copy number of transgenes. In the electroporation method, transgenic plants are generated by protoplast transformation. During the process cells may get damaged by repeated electric pulses, or ion imbalance and cell death may occur (Husaini et al. 2010). NPs combined with chemical compounds deliver genes into target cells. These delivery systems are highly efficient, size tunable (1-200 nm), functionally customizable, and the immunogenicity of the process can be controlled. As the particle size decreases from micro to nanoscale, the cell wall barrier becomes a minor obstacle, and cell damage can also be minimized. NPs used for these purposes include calcium phosphate, carbon, silica, gold magnetite, strontium phosphate. Pore enlargement and multifunctionalization of mesoporous silica NPs could facilitate target-specific delivery of proteins, nucleotides and chemicals in plant biotechnology (Torney et al. 2007).

Effects of NPs on plant photosynthetic characteristics

Foliar application

After foliar and soil fertilization with Fe_2O_3 NPs, plants showed positive effect on root elongation and photosynthetic parameters when compared to their bulk counterparts. No adverse impacts on the physiological performance at any growth stage were reported after application of Fe_2O_3 NPs (Alidoust and Isoda 2013). The effect of NPs can increase the photosynthetic rates, which in turn increases the stomatal opening rather than increased CO_2 uptake activity at the chloroplast level. Foliar application of NPs showed positive effects when compared to soil treatment.

Hydroponic application

Silicon improves canopy photosynthesis and its accumulation in plants is controlled by the ability of roots to take up

Si (Ma and Yamaji 2006). The efficiency of SiO_2 NPs shows better performance than bulk silica with regard to germination percentage (GP %), and root growth parameters in maize (Suriyaprabha et al. 2012). We also observed better photosynthetic characteristics, and increased transpiration rate, leading to cooler leaf temperatures in the presence of SiO_2 NPs in hydroponically grown wheat seedlings (Fig. 13).

Concluding remarks

NPs offer a wide range of new and exciting possibilities for already existing laboratory and clinical techniques, as well as for the development of novel methods. However, in order to curb concerns about toxicity issues, detailed and long-term research is required to further characterize NPs that are introduced or inadvertently come into contact with biological systems.

Acknowledgement

This work was supported by the TÁMOP-4.1.1.C-13/1/KONV-2014-0001 program entitled „Practice-oriented, student-friendly modernization of the biomedical education for strengthening the international competitiveness of the rural Hungarian universities”.

References

- Abbaspour A, Norouz-Sarvestani F, Noori A, Soltani N (2015) Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of *Staphylococcus aureus*. Biosens Bioelectron 68:149-155.
- Abe T, Sakamoto K, Kamohara H, Hirano YI, Kuwahara N,

- Ogawa M (1997) Group II phospholipase A2 is increased in peritoneal and pleural effusions in patients with various types of cancer. *Int J Cancer* 74:245-250.
- Alidoust D, Isoda A (2013) Effect of Fe₂O₃ nanoparticles on photosynthetic characteristic of soybean (*Glycine max* (L.) Merr.): foliar spray versus soil amendment. *Acta Physiol Plant* 35:3365-3375.
- Arias JL (2011) Drug targeting strategies in cancer treatment: an overview. *Mini Rev Med Chem* 11:1-17.
- Arias JL, Ruiz MA, Gallardo V, Delgado AV (2008) Tegafur loading and release properties of magnetite/poly(alkylcyanoacrylate) (core/shell) nanoparticles. *J Control Release* 125:50-58.
- Armendariz V, Herrera I, Peralta-Videa JR, Jose-Yacamán M, Troiani H, Santiago P, Gardea-Torresdey JL (2004) Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. *J Nanopart Res* 6:377-382.
- Bansal V, Rautaray D, Bharde A, Ahire K, Sanyal A, Ahmad A, Sastry M (2005) Fungus-mediated biosynthesis of silica and titania particles. *J Mater Chem* 15:2583-2589.
- Batani D, Vinci T, Bleiner D (2013) Laser-ablation and induced nanoparticle synthesis. *Laser Part Beams* 32:1-7.
- Behra R, Krug H (2008) Nanoecotoxicology: Nanoparticles at large. *Nat Nanotechnol* 3:253-254.
- Bennet D, Kim S (2014) Polymer nanoparticles for smart drug delivery. In Demir Sezer A, ed., *Application of Nanotechnology in Drug Delivery*. InTech, Rijeka, 257-310. DOI:10.5772/58422.
- Bentolila LA, Michalet X, Pinaud FF, Dooze S, Li JJ, Sundaresan G, Wu AM, Gambhir SS, Weiss S (2005) Quantum dots for molecular imaging and cancer medicine. *Discov Med* 5:213-218.
- Bo Y, Jin C, Liu Y, Yu W, Kang H (2014) Metabolomic analysis on the toxicological effects of TiO₂ nanoparticles in mouse fibroblast cells: from the perspective of perturbations in amino acid metabolism. *Toxicol Mech Methods* 24:461-469.
- Bondarenko O, Juganson K, Ivask A, Kasemets K, Mortimer M, Kahru A (2013) Toxicity of Ag, CuO and ZnO nanoparticles to selected environmentally relevant test organisms and mammalian cells *in vitro*: a critical review. *Arch Toxicol* 87:1181-1200.
- Bruchez M, Moronne M, Gin P, Weiss S, Alivisatos AP (1998) Semiconductor nanocrystals as fluorescent biological labels. *Science* 281:2013-2016.
- Burchardt AD, Carvalho RN, Valente A, Nativo P, Gilliland D, Garc CP, Passarella R, Pedroni V, Lettieri T (2012) Effects of silver nanoparticles in diatom *Thalassiosira pseudonana* and Cyanobacterium *Synechococcus* sp. *Environ Sci Technol* 46:11336-11344.
- Cech TR (2004) RNA finds a simpler way. *Nature* 428:263-264.
- Chang E, Miller JS, Sun J, Yu WW, Colvin VL, Drezek R, West JL (2005) Protease-activated quantum dot probes. *Biochem Biophys Res Commun* 334:1317-1321.
- Chang Y, Ho G, Kim TJ, Chae KS (2013) Toxicity of magnetic resonance imaging agents: small molecule and nanoparticle. *Curr Top Med Chem* 13:434-445.
- Chatterjee DK, Diagaradjane P, Krishnan S (2011) Nanoparticle-mediated hyperthermia in cancer therapy. *Ther Deliv* 2(8):1001-1014.
- Chen H, Yada R (2011) Nanotechnologies in agriculture: new tools for sustainable development. *Trends Food Sci Tech* 22:585-594.
- Cherchi C, Chernenko T, Diem M, Gu AZ (2011) Impact of nano titanium dioxide exposure on cellular structure of *Anabaena variabilis* and evidence of internalization. *Environ Toxicol Chem* 30:861-869.
- Choi HS, Liu W, Misra P, Tanaka E, Zimmer JP, Itty Ipe B, Bawendi MG, Frangioni JV (2007) Renal clearance of quantum dots. *Nat Biotechnol* 25:1165-1170.
- Choi J, Wang NS (2011) Nanoparticles in biomedical applications and their safety concerns. In Fazel R, ed., *Biomedical Engineering - From Theory to Applications*. InTech, Rijeka, 299-314. DOI:10.5772/18452.
- Ciofani G, Riggio C, Raffa V, Mencias A, Cuschieri A (2009) A bi-modal approach against cancer: magnetic alginate nanoparticles for combined chemotherapy and hyperthermia. *MedHypotheses* 73:80-82.
- Colson P, Henrist C, Cloots R (2013) Nanosphere lithography: a powerful method for the controlled manufacturing of nanomaterials. *J Nanomater* DOI:10.1155/2013/948510.
- Dameron CT, Smith BR, Winge DR (1989) Glutathione-coated cadmium-sulfide crystallites in *Candida glabrata*. *J Biol Chem* 264:17355-17360.
- Darroudi M, Ahmad MB, Zak AK, Zamiri R, Hakimi M (2011) Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-Light. *Int J Mol Sci* 12:6346-6356.
- Demir E, Aksakal S, Turna F, Kaya B, Marcos R (2015) *In vivo* genotoxic effects of four different nano-sizes forms of silica nanoparticles in *Drosophila melanogaster*. *J Hazard Mater* 283:260-266.
- Dewez D, Oukarroum A (2012) Silver nanoparticles toxicity effect on photosystem II photochemistry of the green alga *Chlamydomonas reinhardtii* treated in light and dark conditions. *Toxicol Environ Chem* 94:1536-1546.
- Dhayananthaprabhu J, Lakshmi Narayanan R, Thiyagarajan K (2013) Facile synthesis of gold (Au) nanoparticles using *Cassia auriculata* flower extract. *Adv Mater Res* 678:12-16.
- Dhillon NK, Kalia A, Gosal SS (2012) Effect of biologically synthesized silica nanoparticles on germination of maize and rice varieties. *J Nanomedic Nanotechnol* 3(1):129.
- Dong S, Tang C, Zhou H, Zhao H (2004) Photochemical

- synthesis of gold nanoparticles by the sunlight radiation using a seeding approach. *Gold Bull* 37:187-195.
- Dowlati A, Loo M, Bury T, Fillet G, Beguin Y (1997) Soluble and cell-associated transferrin receptor in lung cancer. *Br J Cancer* 75:1802-1806
- Dubey PK, Mishra V, Jain S, Mahor S, Vyas SP (2004) Liposomes modified with cyclic RGD peptide for tumor targeting. *J Drug Target* 12:257-264.
- Eichert T, Kurtz A, Steiner U, Goldbach HE (2008) Size exclusion limits and lateral heterogeneity of the stomatal foliar uptake pathway for aqueous solutes and water-suspended nanoparticles. *Physiol Plant* 134:151-160.
- Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin C (1997) Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* 277:1078-1081.
- Elrod DB, Partha R, Danila D, Casscells SW, Conyers JL (2009) An iodinated liposomal computed tomographic contrast agent prepared from a diiodophosphatidylcholine lipid. *Nanomedicine* 5:42-45.
- Fan C, Hu Z, Mustapha A, Lin M (2011) Rapid detection of food- and waterborne bacteria using surface-enhanced Raman spectroscopy coupled with silver nanosubstrates. *Appl Microbiol Biot* 92:1053-1061.
- Fan W, Shen B, Bu W, Zheng X, He Q, Cui Z, Zhao K, Zhang S, Shi J (2015) Design of an intelligent sub-50 nm nuclear-targeting nanotheranostic system for imaging guided intranuclear radiosensitization. *Chem Sci* 6:1747-1753.
- Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan D, Langer R (2004) Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res* 64:7668-7672.
- Fattal E, Couvreur P, Dubernet C (2004) "Smart" delivery of antisense oligonucleotides by anionic pH-sensitive liposomes. *Adv Drug Deliv Rev* 56:931-946.
- Felton C, Karmakar A, Gartia Y, Ramidi P, Biris AS, Ghosh A (2014) Magnetic nanoparticles as contrast agents in biomedical imaging: recent advances in iron- and manganese-based magnetic nanoparticles. *Drug Metab Rev* 46:142-154.
- Fernandez V, Eichert T (2009) Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. *Crit Rev Plant Sci* 28:36-68.
- Fleischer A, Neill MAO, Ehwald R (1999) The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. *Plant Physiol* 121:829-838.
- Garner KL, Keller A (2014) Emerging patterns for engineered nanomaterials in the environment: a review of fate and toxicity studies. *J Nanopart Res* 16:2503.
- Ghaffarian H, Saiedi M, Sayyadnejad MA (2011) Synthesis of ZnO nanoparticles by spray pyrolysis method. *Iran J Chem Chem Eng* 30:1-6.
- Gil ES, Hudson SM (2004) Stimuli-responsive polymers and their bioconjugates. *Prog Polym Sci* 29:1173-1222.
- Gottschalk F, Sondere T, Schols R, Nowack B (2009) Modeled environmental concentrations of engineered nanomaterials for different regions. *Environ Sci Tech* 43:9216-9222.
- Hata M, Chomanee J, Thongyen T, Bao L, Tekasakul S, Tekasakul P, Otani Y, Furuuchi M (2014) Characteristics of nanoparticles emitted from burning of biomass fuels. *J Environ Sci* 26:1913-1920.
- Haverkamp RG, Marshall AT (2009) The mechanism of metal nanoparticle formation in plants: limits on accumulation. *J Nanopart Res* 11:1453-1463.
- Husaini AM, Abidin MZ, Parry GA, Sanghera GS, Murtaza I, Alam T, Srivastava DK, Farooqi H, Khan HN (2010) Vehicles and ways for efficient nuclear transformation in plants. *GM Crops* 1:276-287.
- Imai K, Takaoka A (2006) Comparing antibody and small-molecule therapies for cancer. *Nat Rev Cancer* 6:714-727.
- Ivask A, Elbadawy A, Kaweeteerawat C, Boren D, Fischer H, Ji Z, Chang CH, Liu R, Tolaymat T, Telesca D, Zink JJ, Cohen Y, Holden PA, Godwin H (2014) Toxicity mechanisms in *Escherichia coli* vary for silver nanoparticles and differ from ionic silver. *ACS Nano* 8:374-386.
- Jensen SS, Andresen TL, Davidsen J, Hoyrup P, Shnyder SD, Bibby MC, Gill JH, Jorgensen K (2004) Secretory phospholipase A2 as a tumor-specific trigger for targeted delivery of a novel class of liposomal prodrug anticancer etherlipids. *Mol Cancer Ther* 3:1451-1458.
- Jeynes JC, Jeynes C, Merchant MJ, Kirkby KJ (2013) Measuring and modelling cell-to-cell variation in uptake of gold nanoparticles. *Analyst* 138:7070-7074.
- Johnston HJ, Hutchison GR, Christensen FM, Aschberger K, Stone V (2010) The biological mechanisms and physicochemical characteristics responsible for driving fullerene toxicity. *Toxicol Sci* 114:162-182.
- Jones E, Thrall D, Dewhurst MW, Vujaskovic Z (2006) Prospective thermal dosimetry: the key to hyperthermia's future. *Int J Hyperthermia* 22:247-253.
- Karant H, Murthy RSR (2007) pH-sensitive liposomes-principle and application in cancer therapy. *J Pharm Pharmacol* 59:469-483.
- Kim K, Shin KS (2011) Surface-enhanced Raman Scattering: a powerful tool for chemical identification. *Anal Sci* 27:775-783.
- Kim MI, Kim J, Lee J, Jia H, Na HB, Youn JK, Kwak JH, Dohnalkova A, Grate JW, Wang P, Hyeon T (2007) Cross-linked enzyme aggregates in hierarchically-ordered mesoporous silica: a simple and effective method for enzyme stabilization. *Biotechnol Bioeng* 96:210-218.
- Kittler S, Greulich C, Diendorf J, Köller M, Eppel M (2010) Toxicity of silver nanoparticles increases during storage

- because of slow dissolution under release of silver ions. *Chem Mater* 22:4548-4554.
- Kong G, Braun RD, Dewhirst MW (2000) Hyperthermia enables tumor-specific nanoparticle delivery: effect of particle size. *Cancer Res* 60:4440-4445.
- Kouassi GK, Irudayaraj J, McCarty G (2005) Examination of cholesterol oxidase attachment to magnetic nanoparticles. *J Nanobiotechnology* 3:1.
- Koromilas ND, Lainioti GC, Gialeli C, Barbouri D, Kouravelou KB, Karamanos NK, Voyiatzis G, Kallitsis JK (2014) Preparation and toxicological assessment of functionalized carbon nanotube-polymer hybrids. *PloS One* 9:e107029.
- Lammertink B, Deckers R, Storm G, Moonen C, Bos C (2015) Duration of ultrasound-mediated enhanced plasma membrane permeability. *Int J Pharm* 482:92-98.
- Landsiedel R, Ma-Hock L, Hofmann T, Wiemann M, Strauss V, Treumann S, Wohlleben W, Gröters S, Wiench K, van Ravenzwaay B (2014) Application of short-term inhalation studies to assess the inhalation toxicity of nanomaterials. *Part Fibre Toxicol* 11:16.
- Lara HH, Garza-Trevino EN, Ixtapan-Turrent L, Singh DK (2011) Silver nanoparticles are broad-spectrum bactericidal and virucidal compounds. *J Nanobiotechnology* 9:30.
- Lavanya V, Adil AAM, Ahmed N, Jamal S (2014) Lectins - the promising cancer therapeutics. *Oncobiol Targets* 1:12-15.
- Lee JH, Choi SUS, Jang S, Lee S (2012) Production of aqueous spherical gold nanoparticles using conventional ultrasonic bath. *Nanoscale Res Lett* 7:420.
- Lehto M, Karilainen T, Róg T, Cramariuc O, Vanhala E, Tornaes J, Taberman H, Janis J, Alenius H, Vattulainen I, Laine O (2014) Co-exposure with fullerene may strengthen health effects of organic industrial chemicals. *PloS One* 9:e114490.
- Lengke MF, Fleet ME, Southam G (2006) Morphology of gold nanoparticles synthesized by filamentous cyanobacteria from gold(I)-thiosulfate and gold(III)-chloride complexes. *Langmuir* 22:2780-2787.
- Li D, He Q, Cui Y, Duan L, Li J (2007) Immobilization of glucose oxidase onto gold nanoparticles with enhanced thermostability. *Biochem Biophys Res Commun* 355:488-493.
- Li Y, Zhang W, Niu J, Chen Y (2013) Surface-coating-dependent dissolution, aggregation, and reactive oxygen species (ROS) generation of silver nanoparticles under different irradiation conditions. *Environ Sci Technol* 47:10293-10301.
- Lidke DS, Nagy P, Heintzmann R, Arndt-Jovin DJ, Post JN, Grecco HE, Jares-Erijman E, Jovin TM (2004) Quantum dot ligands provide new insights into erbB/HER receptor-mediated signal transduction. *Nat Biotechnol* 22:198-203.
- Lin D, Tang T, Jed Harrison D, Lee WE, Jemere AB (2015) A regenerating ultrasensitive electrochemical impedance immunosensor for the detection of adenovirus. *Biosens Bioelectron* 68:129-134.
- Liu J, Lu Y (2005) Stimuli-responsive disassembly of nanoparticle aggregates for light-up colorimetric sensing. *J Am Chem Soc* 127:12677-12683.
- Liu W, Zhang S, Wang P (2009) Nanoparticle-supported multi-enzyme biocatalysis with in situ cofactor regeneration. *J Biotechnol* 139:102-107.
- Loginova YF, Dezhurov SV, Zherdeva VV, Kazachkina NI, Wakstein MS, Savitsky AP (2012) Biodistribution and stability of CdSe core quantum dots in mouse digestive tract following per os administration: advantages of double polymer/silica coated nanocrystals. *Biochem Biophys Res Commun* 419:54-59.
- Lundqvist M, Stigler J, Elia G, Lynch I, Cedervall T, Dawson KA (2008) Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proc Natl Acad Sci USA* 105:14265-14270.
- Ma JF, Yamaji N (2006) Silicon uptake and accumulation in higher plants. *Trends Plant Sci* 11:392-397.
- Ma Y, Kuang L, He X, Bai W, Ding Y, Zhang Z, Zhao Y, Chai Z (2010) Effects of rare earth oxide nanoparticles on root elongation of plants. *Chemosphere* 78:273-279.
- Maillard JY, Hartemann P (2012) Silver as an antimicrobial: facts and gaps in knowledge. *Crit Rev Microbiol* 39:1-11.
- Makarov VV, Love AJ, Sinitsyna OV, Makarova SS, Yaminsky IV, Taliansky ME, Kalinina NO (2014) "Green" nanotechnologies: synthesis of metal nanoparticles using plants. *Acta Naturae* 6:35-44.
- Marambio-Jones C, Hoek EMV (2010) A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. *J Nanopart Res* 12:1531-1551.
- Marchiol L, Mattiello A, Poscic F, Giordano C, Musetti R (2014) *In vivo* synthesis of nanomaterials in plants: location of silver nanoparticles and plant metabolism. *Nanoscale Res Lett* 9:101.
- Matorin DN, Todorenko D, Seifullina NK, Zayadan BK, Rubin AB (2014) Effect of silver nanoparticles on the parameters of chlorophyll fluorescence and P700 reaction in the green alga *Chlamydomonas reinhardtii*. *Microbiology* 82:809-814.
- Maxwell DJ, Taylor JR, Nie S (2002) Self-assembled nanoparticle probes for recognition and detection of biomolecules. *J American Chem Soc* 124:9606-9612.
- Michalet X (2005) Quantum dots for live cells, *in vivo* imaging, and diagnostics. *Science* 307:538-544.
- Mijnendonckx K, Leys N, Mahillon J, Silver S, Van Houdt R

- (2013) Antimicrobial silver: uses, toxicity and potential for resistance. *BioMetals* 26:609-621.
- Miller RJ, Bennett S, Keller A, Pease S, Lenihan HS (2012) TiO₂ nanoparticles are phototoxic to marine phytoplankton. *PLoS One* 7: e30321.
- Miranda OR, Creran B, Rotello VM (2010) Array-based sensing with nanoparticles: 'chemical noses' for sensing biomolecules and cell surfaces. *Curr Opin Chem Biol* 14:728-736.
- Moncada E, Quijada R, Retuert J (2007) Nanoparticles prepared by the sol-gel method and their use in the formation of nanocomposites with polypropylene. *Nanotechnology* 18:335606.
- Monica RC, Cremonini R (2009) Nanoparticles and higher plants. *Caryologia* 62:161-165.
- Mukherjee P, Ahmad A, Mandal D, Senapati S, Sainkar SR, Khan MI, Parishcha R, Ajaykumar PV, Alam M, Kumar R, Sastry M (2001) Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel biological approach to nanoparticle synthesis. *Nano Lett* 1:515-519.
- Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS (2010) Nanoparticulate material delivery to plants. *Plant Sci* 179:154-163.
- Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao AJ, Quigg A, Santschi PH, Sigg L (2008) Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology* 17:372-386.
- Nel A, Xia T, Madler L, Li N (2006) Toxic potential of materials at the nanolevel. *Science* 311:622-627.
- Oberdörster G, Oberdörster E, Oberdörster J (2005) Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. *Environ Health Perspect* 113:823-839.
- OECD (2012) Important issues on risk assessment of manufactured nanomaterials. Available: <http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono%282012%29&doclanguage=en>
- Oomen AG, Bos PMJ, Fernandes TF, Hund-Rinke K, Boraschi D, Byrne HJ, Aschberger K, Gottardo S, von der Kammer F, Kühnel D, Hristozov D, Marcomini A, Migliore L, Scott-Fordsmand J, Wick P, Landsiedel R (2014) Concern-driven integrated approaches to nanomaterial testing and assessment—report of the NanoSafety Cluster Working Group 10. *Nanotoxicology* 8:334-348.
- Oukarroum A, Polchtchikov S, Perreault F, Popovic R (2012) Temperature influence on silver nanoparticles inhibitory effect on photosystem II photochemistry in two green algae, *Chlorella vulgaris* and *Dunaliella tertiolecta*. *Environ Sci Pollut Res* 19:1755-1762.
- Pandey P, Singh SP, Arya SK, Gupta V, Datta M, Singh S, Malhotra BD (2007) Application of thiolated gold nanoparticles for the enhancement of glucose oxidase activity. *Langmuir* 23:3333-3337.
- Pasqualini R, Koivunen E, Ruoshlati E (1997) Alpha v integrins as receptors for tumor targeting by circulating ligands. *Nat Biotechnol* 15:542-546.
- Perrin T, Hemett OM, Menth M, Descombes E (2012) Contrast-induced acute kidney injury following iodine opacification other than by intravascular injection. *Clin Kidney J* 5:456-458.
- Pestourie C, Tavitian B, Duconge F (2005) Aptamers against extracellular targets for *in vivo* applications. *Biochimie* 87:921-930.
- Phenix CP, Togtema M, Pichardo S, Zehbe I, Curiel L (2014) High intensity focused ultrasound technology, its scope and applications in therapy and drug delivery. *J Pharm Pharm Sci* 17:136-153.
- Pillai S, Behra R, Nestler H, Suter MJF, Sigg L, Schirmer K (2014) Linking toxicity and adaptive responses across the transcriptome, proteome, and phenotype of *Chlamydomonas reinhardtii* exposed to silver. *Proc Natl Acad Sci USA* 111:3490-3495.
- Planchon M, Jittawuttipoka T, Cassier-Chauvat C, Guyot F, Gelabert A, Benedetti MF, Chauvat F, Spalla O (2013) Exopolysaccharides protect *Synechocystis* against the deleterious effects of titanium dioxide nanoparticles in natural and artificial waters. *J Colloid Interface Sci* 405:35-43.
- Rai M, Yadav A, Gade A (2009) Silver nanoparticles as a new generation of antimicrobials. *Biotechnol Adv* 27:76-83.
- Reidy B, Haase A, Luch A, Dawson K, Lynch I (2013) Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications. *Materials* 6:2295-2350.
- Ren Y, Rivera JG, He L, Kulkarni H, Lee DK, Messersmith PB (2011) Facile, high efficiency immobilization of lipase enzyme on magnetic iron oxide nanoparticles via a biomimetic coating. *BMC Biotechnol* 11:63.
- Shankar SS, Rai A, Ahmad A, Sastry M (2004) Rapid synthesis of Au, Ag, and bimetallic Au core-Ag shell nanoparticles using Neem (*Azadirachta indica*) leaf broth. *J Colloid Interface Sci* 275:496-502.
- Sharp DG, Taylor AR, Mclean IW, Beard JD, Beard JW (1945) Densities and sizes of the influenza viruses A (PR8 strain) and B (LEE strain) and the swine influenza virus. *J Biol Chem* 159:29-44.
- Sibag M, Choi BG, Suh C, Lee KH, Lee JW, Maeng SK, Cho J (2015) Inhibition of total oxygen uptake by silica nanoparticles in activated sludge. *J Hazard Mater* 283:841-846.
- Silva LG, Solis-Pomar F, Gutierrez-Lazos CD, Melendrez MF, Martinez E, Fundora A, Perez-Tijerina E (2014) Synthesis of Fe nanoparticles functionalized with oleic acid synthesized by inert gas condensation. *J Nanomater*

- 2014:Art ID 643967.
- Simoës S, Nuno Moreira J, Fonseca C, Düzgünes N, Pedroso De Lima MC (2004) On the formulation of pH-sensitive liposomes with long circulation times. *Adv Drug Deliv Rev* 56: 947-965.
- Singh S, D'Britto V, Bharde A, Sastry M, Dhawan A, Prasad BLV (2010) Bacterial synthesis of photocatalytically active and biocompatible TiO₂ and ZnO nanoparticles. *Int J Green Nanotechnol Phys Chem* 2:80-99.
- Sommaruga S, Galbiati E, Penaranda-Avila J, Brambilla C, Tortora P, Colombo M, Prosperi D (2014) Immobilization of carboxypeptidase from *Sulfolobus solfataricus* on magnetic nanoparticles improves enzyme stability and functionality in organic media. *BMC Biotechnol* 14:82.
- Stipe CB, Choi JH, Lucas D, Koshland CP, Sawyer RF (2004) Nanoparticle production by UV irradiation of combustion generated soot particles. *J Nanopart Res* 6:467-477.
- Sudimack J, Lee RJ (2000) Targeted drug delivery via the folate receptor. *Adv Drug Deliv Rev* 41:147-162.
- Sun TY, Gottschalk F, Hungerbühler K, Nowack B (2014) Comprehensive probabilistic modelling of environmental emissions of engineered nanomaterials. *Environ Pollut* 185:69-76.
- Sundaram J, Park B, Kwon Y, Lawrence KC (2013) Surface enhanced Raman scattering (SERS) with biopolymer encapsulated silver nanosubstrates for rapid detection of foodborne pathogens. *Int J Food Microbiol* 167:67-73.
- Suriyaprabha R, Karunakaran G, Yuvakkumar R, Rajendran V, Kannan N (2012) Silica nanoparticles for increased silica availability in maize (*Zea mays*. L) seeds under hydroponic conditions. *Curr Nanosci* 8:902-908.
- Ta T, Bartolak-Suki E, Park EJ, Karrobi K, McDannold NJ, Porter TM (2014) Localized delivery of doxorubicin in vivo from polymer-modified thermosensitive liposomes with MR-guided focused ultrasound-mediated heating. *J Control Release* 194:71-81.
- Tang SH (2010) Nanoparticle-based immunoassays for sensitive and early detection of HIV-1 capsid (p24) antigen. *J Infect Dis* 201:S59-S64.
- Tannock IF, Rotin D (1989) Acid pH in tumors and its potential for therapeutic exploitation. *Cancer Res* 49:4373-4384.
- Tedetti M, Sempere R (2006) Penetration of ultraviolet radiation in the marine environment. A Review. *Photochem Photobiol* 82:389-397.
- Tepe N, Bau M (2014) Importance of nanoparticles and colloids from volcanic ash for riverine transport of trace elements to the ocean: evidence from glacial-fed rivers after the 2010 eruption of Eyjafjallajökull Volcano, Iceland. *Sci Total Environ* 488-489:243-251.
- Thakker JN, Dalwadi P, Dhandhukia PC (2013) Biosynthesis of gold nanoparticles using *Fusarium oxysporum* f. sp. *cubense* JT1, a plant pathogenic fungus. *ISRN Biotechnology* 2013:Art ID 515091.
- Tilly TB, Kerr LL, Braydich-Stolle LK, Schlager JJ, Hussain SM (2014) Dispersions of geometric TiO₂ nanomaterials and their toxicity to RPMI 2650 nasal epithelial cells. *J Nanopart Res* 16:2695.
- Torchilin V (2011) Tumor delivery of macromolecular drugs based on the EPR effect. *Adv Drug Deliver Rev* 63:131-135.
- Torchilin V (2006) Multifunctional nanocarriers. *Adv Drug Deliver Rev* 58:1532-1555.
- Torney F, Trewyn BG, Lin VSY, Kan W (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. *Nat Nanotechnol* 2:295-300.
- Tu Y, Lv M, Xiu P, Huynh T, Zhang M, Castelli M, Liu Z, Huang Q, Fan C, Fang H, Zhou R (2013) Destructive extraction of phospholipids from *Escherichia coli* membranes by graphene nanosheets. *Nat Nanotechnol* 8:594-601.
- Udaya Prakash NK, Bhuvaneswari S, Prabha SB, Kavitha K, Sandhya KV, Sathyabhuvaneswari P, Bharathiraja (2014) Green synthesis of silver nanoparticles using airborne Actinomycetes. *Int J ChemTech Res* 6:4123-4127.
- Udey MC, Chaplin DD, Wedner HJ, Parker CW (1980) Early activation events in lectin-stimulated human lymphocytes: evidence that wheat germ agglutinin and mitogenic lectins cause similar early changes in lymphocyte metabolism. *J Immunol* 125:1544-1550.
- Urano M, Kuroda M, Nishimura Y (1999) For the clinical application of thermochemotherapy given at mild temperatures. *Int J Hyperthermia* 2:79-107.
- Vigor KL, Kyrtatos PG, Minogue S, Al-Jamal KT, Kogelberg H, Tolner B, Kostarelos K, Begent RH, Pankhurst Q, Lythgoe MF, Chester K (2010) Nanoparticles functionalized with recombinant single chain Fv antibody fragments (scFv) for the magnetic resonance imaging of cancer cells. *Biomaterials* 31:1307-1315.
- Verano-Braga T, Miethling-Graff R, Wojdyla K, Rogowska-Wrzesinska A, Brewer JR, Erdmann H, Kjeldsen F (2014) Insights into the cellular response triggered by silver nanoparticles using quantitative proteomics. *ACS Nano* 8:2161-2175.
- von Moos N, Slaveykova VI (2014) Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae--state of the art and knowledge gaps. *Nanotoxicology* 8:605-630.
- Wang ZM, Wagner J, Wall S (2011) Characterization of Laser Printer Nanoparticle and VOC Emissions, Formation Mechanisms, and Strategies to Reduce Airborne Exposures. *Aerosol Sci Tech* 45:1060-1068.
- Ward MB, Brydson R, Cochrane RF (2006) Mn nanoparticles produced by inert gas condensation. *J Phys: Conf Ser* 26:296-299.

- Wei C, Zhang Y, Guo J, Han B, Yang X, Yuan J (2010) Effects of silica nanoparticle on growth and photosynthetic pigment contents of *Scenedesmus obliquus*. J Environ Sci 22:155-160.
- Xu ZP, Kurniawan ND, Bartlett PF, Lu GQ (2007) Enhancement of relaxivity rates of Gd-DTPA complexes by Intercalation into layered double hydroxide nanoparticles. Chemistry 13:2824-2830.
- Yah CS, Iyuke SE, Simate GS (2011) A review of nanoparticles toxicity and their routes of exposures. Iran J Pharm Sci 8:299-314.
- Yu Y, Duan J, Yu Y, Li Y, Liu X, Zhou X, Ho KF, Tian L, Sun Z (2014) Silica nanoparticles induce autophagy and autophagic cell death in HepG2 cells triggered by reactive oxygen species. J Hazard Mater 270:176-186.
- Zhang T, Tang M, Kong L, Li H, Zhang T, Xue Y, Pu Y (2015) Surface modification of multiwall carbon nanotubes determines the pro-inflammatory outcome in macrophage. J Hazard Mater 284:73-82.
- Zhang Y (2003) Global non-viral gene transfer to the primate brain following intravenous administration. Mol Ther 7:11-18.
- Zharov VP, Mercer KE, Galitovskaya EN, Smeltzer MS (2006) Photothermal nanotherapeutics and nanodiagnostics for selective killing of bacteria targeted with gold nanoparticles. Biophys J 90:619-627.
- Zhou J, Sun Y, Du X, Xiong L, Hu H, Li F (2010) Dual-modality in vivo imaging using rare-earth nanocrystals with near-infrared to near-infrared (NIR-to-NIR) upconversion luminescence and magnetic resonance properties. Biomaterials 31:3287-3295.
- Zhu Y, Hong H, Xu ZP, Li Z, Cai W (2013) Quantum dot-based nanoprobe *in vivo* targeted imaging. Curr Mol Med 13:1549-1567.
- Zimmer AT, Biswas P (2001) Characterization of the aerosols resulting from arc welding processes. J Aerosol Sci 32:993-1008.